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The processes of mammary gland development and mammary tumor growth require extensive remodeling of the extracellular matrix (ECM). Proteins that degrade the ECM such as matrix metalloproteinases (MMPs), are important during these and several other matrix degrading processes. We hypothesized that an epithelial specific MMP, matrilysin (*MAT*), is partly responsible for remodeling of the ECM during mammary development and tumorigenesis. To test our hypothesis, transgenic mice expressing *MAT* under the control of the mouse mammary tumor virus (MMTV) promoter/enhancer were generated to investigate if overproduction of *MAT* alters mammary development and/or mammary tumorigenesis. In this report we show that although overexpression of *MAT* does not alter normal mammary development, the presence of *MAT* does induce premature milk production in normal transgenic mammary glands. In addition, the expression of *MAT* in MMTV-*Mat/neu* double transgenic animals accelerates the onset and frequency of mammary tumorigenesis.

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FOREWORD

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Lana A. Rudolph 9/1/97

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION

Cell-matrix interactions are an important aspect to many biological processes. During processes such as mammary growth and neoplasia the extracellular matrix (ECM) is continuously degraded and remodeled. Proteins that degrade the extracellular matrix, such as matrix metalloproteinases (MMPs), clearly play a role in the interactions that occur within the extracellular environment. We hypothesize that matrilysin (*MAT*), an epithelial specific MMP, is partly responsible for remodeling of the ECM during mammary development and tumorigenesis. To test our hypothesis, transgenic mice expressing *MAT* under the control of the mouse mammary tumor virus (MMTV) promoter/enhancer have been evaluated to investigate if overproduction of *MAT* alters mammary development and/or mammary tumorigenesis.

Our first annual report described the successful generation of transgenic animals that express native, active, and inactive human *MAT* under the control of the MMTV promoter in the mammary epithelium (*specific aim #1*). For review, three separate human *MAT* constructs were used to develop three different transgenic lines: 1) a native, or wild-type transgene, 2) a constitutively activated transgene, and 3) an inactive *MAT* transgene. The constitutively active construct contains a mutation that results in spontaneous activation of the enzyme, therefore circumventing any dependence on activation by exogenous factors. A comparison of the results from the native and active *MAT* constructs should give an indication of the availability of activators of matrilysin in the mammary environment. The third construct encodes a MAT protein that lacks proteolytic activity due to the presence of an inactivating mutation. The use of this mutant should determine if any observed effect of *MAT* in this model is due to its proteolytic activity. In this final report we will present results obtained from the completed characterization of these transgenic animals.

Our second annual report addressed whether mammary tumorigenesis can be modified by overexpression of native *MAT* in induced mammary tumors (*specific aim #2*). To address this question, we mated mice expressing the wild-type MAT protein with animals expressing the oncogene *neu* under the control of the MMTV promoter (Guy et al, 1992). *neu*/c-erbB2 has been observed to be amplified and overexpressed in a significant number of human breast cancers (Slamon et al, 1987). Overexpression of the *neu* product in the murine mammary epithelium results in the appearance of focal mammary tumors in multiparous females by approximately 205 days that metastasized to the lungs in 70% of tumor bearing animals (Guy et al, 1992).

Transgenic mice expressing wild-type MAT and neu protein under the control of the MMTV promoter were analyzed for the time and frequency of onset, growth rate, and presence of metastasis. Our data indicated that the *MAT /neu* females develop mammary tumors at an accelerated rate and higher frequency than the *neu* control females. However, we observed no obvious difference in the growth rate or the development of metastasis in the *MAT /neu* mice when compared to the *neu* controls. These results are remarkably similar to results

recently obtained by Drs. R.J. Coffey, Jr., Vanderbilt University, and W. Muller, McMaster University, in which they crossed the MMTV-*TGFα* (Matsui et al, 1990) transgenics with the MMTV-*neu* mice (Guy et al, 1992) and also observed significant acceleration in the onset of tumor development (Muller et al, 1996). These results led us to the hypothesis that there may be a connection between *MAT* and the EGF/ErbB receptor signal transduction pathway that is related to accelerated mammary tumor growth. This final report outlines experiments that test this hypothesis.

FINAL REPORT - PROGRESS

A. Consequence of *MAT* overexpression in the normal murine mammary gland.

For review of our previous annual reports, we have generated three distinct *MAT* expression constructs to produce wildtype, constitutively active, and inactive forms of the MAT protein. The MMTV-MAT expression vectors producing the MAT proteins were used to establish transgenic mouse lines by standard pronuclear injection techniques. The resulting transgenic lines were identified by the founder animal number and will be referred to hereafter as MMTV-*MAT* (MMTV-wildtype-matrilysin), MMTV-Act*MAT* (MMTV-active-matrilysin), and MMTV-In*MAT* (MMTV-inactive-matrilysin).

The MMTV-LTR promoter/enhancer has been used extensively to drive the expression of transgenes in the mammary epithelium (Cardiff and Muller, 1993 for review). The MMTV-LTR promoter activity responds to endogenous steroid hormone levels in the murine mammary glands during development, pregnancy and lactation, as well as during the normal estrous cycle (Gunzburg and Salmons, 1992). As previously outlined, *MAT* expression in the transgenic animals was analyzed by northern blot analysis of poly(A+) RNA from developing mammary glands of female mice harboring the wildtype, active, and inactive human *MAT* constructs (Figure 1). We observed considerable variability in the expression of the *MAT* transgene within and between the various transgenic lines, presumably due to hormonal fluctuations. For example, approximately 42% (8/19) of transgenic mammary glands examined from MMTV-*MAT* line #3 expressed human *MAT* wildtype mRNA at various stages of mammary development. No correlation of human *MAT* mRNA expression could be made for a particular time during mammary development or during a specific day of the estrous cycle. Human *MAT* expression did appear to be abundant between 6 and 17 weeks of age in the MMTV-*MAT* line #3 and #42, and was absent in nontransgenic littermate controls (Figure 1A). MMTV-Act*MAT* line #1 and #22 also displayed detectable levels of *MAT* mRNA during mammary gland development, although in general *MAT* mRNA levels appeared lower than for MMTV-*MAT* mice (Figure 1B). In contrast, *MAT* mRNA appeared as a smear instead of a distinct band when isolated from the mammary glands of both the MMTV-In*MAT* line #2 and line #4 animals (Figure 1C). Several attempts were made to extract intact human *MAT* RNA from the

mammary glands of the MMTV-In*MAT* transgenic lines, all of which proved to be futile.

Immunohistochemistry was performed to localize the product of the MAT transgene in the mammary glands. Protein expression from the MMTV-*MAT* wildtype transgene was detected during several stages of mammary gland development (6 to 14 weeks), with staining localized to the cytoplasm of the epithelial cells of the mammary ducts (Figure 2A). MAT protein was also detected in mammary tissue from the MMTV-Act*MAT* lines, but at relatively lower levels than the MMTV-*MAT* wildtype lines (Figure 2B). We detected no MAT immunoreactivity in mammary glands from the MMTV-In*MAT* animals, suggesting that the mutation impairs the production of MAT protein in vivo (Figure 2C). No immunoreactivity was detected in any mammary gland sections from nontransgenic littermate controls at various times of mammary development (Figure 2D). Because of the absence or low expression of MAT protein in the transgenic animals carrying the mutated human *MAT* cDNA constructs and the high *MAT* expression in the transgenic animals carrying the wildtype human *MAT* cDNA construct, we focused our attention in subsequent studies on those animals carrying the wildtype *MAT* transgene. In general, initial observations suggest that transgenic animals carrying the activated form of *MAT* showed similar phenotypes to wildtype *MAT* transgenics but to a lesser degree, while the inactive *MAT* transgenics were indistinguishable from nontransgenic controls.

Previous studies have indicated that the expression of the MMP stromelysin-1 (*STR-1*) in mammary epithelial cells results in disruption of the basement membrane and subsequent changes in the proliferative and apoptotic indices of these cells, as well as premature lobuloalveolar development and milk protein production in virgin female mice (Sympson et al, 1994; Witty et al, 1995b). Therefore, we were interested in investigating the effects of overexpressing the epithelium-specific MMP *MAT* in the epithelial cells of developing murine mammary glands and comparing the effect to previous results with *STR-1* overexpression.

The ductal tree of developing mammary glands in nontransgenic and MMTV-*MAT* transgenic mice was examined by whole mount tissue preparation. During mammary gland development, which begins at approximately 5 weeks of age and following the onset of estrogen production, the mammary end buds grow outward from the nipple to fill the entire fat pad with a highly branched network of epithelial cells (Snedeker et al, 1991 and references therein). In contrast to the preliminary data from an isolated animal presented in our first report, we show that there is no apparent morphological difference in the mammary ductal tree during any stage of development in virgin MMTV-*MAT* (Figure 3A and B) when compared to nontransgenic littermate controls (Figure 3C and D). We also observed no difference in mammary gland morphology in the MMTV-Act*MAT* and MMTV-In*MAT* animals (data not shown).

Although the MMTV-*MAT* glands display normal morphology, we examined them for subtle changes in differentiation, proliferation, or apoptosis that may have occurred in response to MMTV-*MAT*. Production of the milk proteins in the casein family is normally restricted to differentiated mammary epithelial cells

during late pregnancy and lactation. However, using an antibody specific for mixed caseins, milk proteins were detected in all virgin *MAT* transgenic animals previously shown to express the *MAT* transgene by northern analysis or MAT protein by immunohistochemistry (Figure 4A and B). No casein was detected in age-matched nontransgenic control mammary glands (Figure 4C and D). These results suggest that there is aberrant differentiation of mammary epithelial cells as a result of the transgene expression, although there are no accompanying morphological changes resembling lobuloalveolar development. The lack of morphological changes is consistent with our inability to detect differences in the number of proliferative or apoptotic cells in the MMTV-*MAT* mammary glands compared to age-matched, nontransgenic controls. We observed no significant difference in the number or location of proliferating cells as determined by immunoreactivity with the cell cycle marker PCNA, or apoptotic cells as determined by the number of cells with excessive nuclear DNA fragmentation (TUNEL assay; data not shown).

B. Overexpression of *MAT* influences the early onset of mammary tumorigenesis.

To investigate a potential role for *MAT* in mammary tumorigenesis, we induced mammary tumors in MMTV-*MAT* transgenic mice by mating them with MMTV-*neu* animals. Bigenic MMTV-*MAT/neu* animals developed mammary tumors with a morphological and histological appearance similar to those previously reported in MMTV-*neu* single transgenic animals. Histological examination of lung tissue from these affected animals revealed the presence of multiple nodular lesions lodged in pulmonary vessels. These lesions were verified as metastases originated from mammary tumors by the presence of β-casein immunoreactivity (Guy et al, 1992, data not shown). The presence of the protein produced from the *MAT* transgene in the MMTV-*MAT/neu* mammary tumors was confirmed using an anti-MAT antibody that reacts with human, but not mouse MAT (Rudolph-Owen et al, 1997). The MAT protein product was detected in isolated groups of cells lying at the periphery of MMTV-*MAT/neu* mammary tumors, and not in tumors derived from MMTV-*neu* only transgenic mice (Figure 5).

Although the *MAT*-expressing mice showed no significant alterations in tumor growth or metastasis, as outlined in the previous annual report, we observed a dramatic acceleration in tumor onset in MMTV-*MAT/neu* mice compared to the MMTV-*neu* control animals. Fifty percent of female bigenic animals developed mammary tumors by approximately 27 weeks, while 50% of single transgenic animals developed mammary tumors by approximately 40 weeks ($p<0.00001$ by a log-rank test, Fig. 2). In addition, 100% of the MMTV-*MAT/neu* double transgenic females formed mammary tumors by 40 weeks of age, whereas 20% of the *neu* females were still tumor-free by 60 weeks of age. Thus, the overexpression of *MAT* in *neu*-expressing mammary glands enhanced tumorgenesis by increasing the frequency of tumor development and shortening the time of tumor onset by an average of 13 weeks.

In the previous report we suggested that the similarities in the accelerated response of MMTV-*neu* tumors to both TGFα and matrilysin may be that matrilysin is responsible for the proteolytic processing of the EGF/erbB tyrosine kinase receptor family and/or their growth factor ligands. In that report, we also presented preliminary data that supported this hypothesis. Unfortunately, further examination of potential processing of the ErbB receptors and/or their growth factor ligands by matrilysin was not substantiated. However, we have analyzed the ability of Neu to heterodimerize and activate other ErbB receptor family members in MMTV-*MAT/neu* and MMTV-*neu* mammary tumors.

Antibodies specific to the EGF receptor, ErbB-3 and ErbB-4 were used to immunoprecipitate these receptors from mammary tumor protein lysates, either as complexes or isolated molecules. The presence of Neu within pre-existing cellular complexes was then analyzed by western blotting. Immunoprecipitation with anti-EGF receptor and subsequent blotting for Neu revealed that Neu protein co-immunoprecipitated with the EGF receptor (Figure 6A for representative samples). In addition, Neu was shown to co-immunoprecipitate with ErbB-3 and ErbB-4 in the same mammary tumor protein lysates (data not shown). There was no discernible difference in the association of Neu with other family member receptors in the MMTV-*MAT/neu* mammary tumors compared to the MMTV-*neu* tumors.

To determine if signalling through ErbB receptors occurred, phosphotyrosine levels (p-Tyr) of these proteins was analyzed by immunoprecipitation with anti-receptor antibody and western blotting with anti-p-Tyr (Fig. 6B and C for representative samples). The EGF receptor was present and phosphorylated at moderate levels in both the MMTV-*MAT/neu* and MMTV-*neu* mammary tumor extracts (Fig. 6B). High levels of Neu were also found in mammary tumors and were associated with high levels of p-Tyr in both sets of tumors (Fig. 6C). ErbB-3 and ErbB-4 were detected within the mammary tumor extracts, but were associated with very low or undetectable levels of p-Tyr (data not shown). These data illustrate that the EGF receptor and Neu are the only ErbB receptor family members that were activated at relatively consistent levels within the mammary tumor extracts. Importantly, the level of activation of the ErbB receptors was similar between the MMTV-*MAT/neu* tumors and the MMTV-*neu* tumors, suggesting that MAT has no effect on the levels of ErbB receptor signalling in fully-developed mammary tumors.

Constitutive activation of *neu* by small deletions in the cytoplasmic domain has been demonstrated to contribute to the development of spontaneous mammary gland tumors observed in the MMTV-*neu* animals (Siegel et al., 1994). Examination of the *neu* transgene by RNAse protection revealed deletions in 67% (4/6) of our MMTV-*MAT/neu* mammary tumor samples, while uninvolved mammary glands from these same animals lacked any *neu* alterations (data not shown). The development of mammary tumors therefore corresponds to mutations in the *neu* transgene in MMTV-*MAT/neu* mice similar to that observed in MMTV-*neu* mice.

Phosphorylation and activation of the ErbB receptors could also presumably occur before the development of the mammary tumors. Mammary glands from MMTV-*MAT/neu* and MMTV-*neu* virgin animals at 25 - 30 weeks of age that were free of mammary tumors were processed and protein extracts examined for the

presence of ErbB receptors and levels of p-Tyr. These data revealed little difference between the expression levels of the ErbB receptors, or their levels of p-Tyr between the MMTV-*MAT/neu* and the MMTV-*neu* alone virgin, tumor free mammary glands.

As previously stated, the overexpression of the *MAT* transgene in MMTV-*MAT* single transgenic female mice does not produce any observable morphological changes during mammary gland development. Careful examination, however, of mammary whole mounts showed that 50% (4/8) of aged, multiparous *MAT* wildtype transgenic females contained abnormal structures in the mammary glands (Fig. 7A), while multiparous non-transgenic mammary glands were devoid of such structures (0/6, Fig. 7B). These distinctive focal areas of epithelial hyperplasia have been previously termed hyperplastic alveolar nodules (HANs), and are considered to be premalignant precursors that are prone to develop into mammary carcinomas (Daniel and Silberstein, 1987; Cardiff, 1984). Previous studies suggest that the HAN is probably derived from a single cell; with each HAN representing a clonal population of cells (Cardiff, 1984). However, it has also been demonstrated that an individual HAN population can undergo further genetic changes that result in a biologically heterogeneous population of hyperplastic cells. For example, when a single HAN is divided and each portion transplanted into a separate fat pad, the resulting outgrowths are morphologically and biologically diverse, demonstrating the pluripotent nature of the HAN population. HANs are also susceptible to carcinogens. Exposure to exogenous hormones, chemical carcinogens, viruses, or radiation increases the tumor incidence of the hyperplastic cells and usually decreases the tumor latency period (Cardiff, 1984). The appearance of HANs in the MMTV-*MAT* mammary glands and not in age-matched and pregnancy-matched nontransgenic animals suggests that the overexpression of *MAT* predisposes to the formation of these preneoplastic lesions.

CONCLUSIONS

The developing murine mammary gland has provided an excellent model system to examine the role of MMPs in a remodeling tissue. The expression patterns of MMPs suggest that they play an important role in the dramatic morphological and functional changes that take place in the mammary gland during ductal development. Overexpression of human MAT protein had no effect on the general morphological development of the mammary ductal tree, but induced the ectopic expression of a pregnancy-associated protein, β-casein, in developing virgin transgenic mammary glands. In contrast, the MMTV-*STR-1* (Witty et al., 1995b) and WAP-*STR-1* (Sympson et al., 1994) transgenic animals express β-casein mRNA, but not protein, display the morphological features of precocious lobuloalveolar development, and demonstrate increased proliferation and apoptotic indices (Witty et al, 1995a; Boudreau et al, 1996). There are several potential explanations for these differences. Experimental variation, such as differences in the integration sites, expression levels, and genetic backgrounds of the

mice may be contributing factors, although the phenotypes were observed in several independent lines of mice in all cases. *STR-1* contains a hemopexin/vitronectin-like domain which is absent in *MAT*, and may confer additional activities or alter substrate specificity in vivo resulting in the observed phenotypic differences. The abnormal tissue-type expression of *STR-1* in glandular epithelial cells, as opposed to the normal expression in stromal fibroblast-like cells surrounding the developing ducts (Witty et al, 1995b) may also account for the more profound cellular alterations in these mice compared to *MAT* transgenic animals. In addition, the differential endogenous expression levels of *STR-1* and *MAT* in the mammary gland suggest that these MMPs may have distinct roles during mammary development. The low endogenous expression levels of *MAT* (Wilson et al, 1995) implies that this particular MMP plays a minor role in mammary development compared to the abundantly expressed *STR-1* (Witty et al, 1995b), which may explain the less dramatic consequences of *MAT* overexpression. Although the morphological features of lobuloalveolar development were not observed in the MMTV-*MAT* transgenic mice, they displayed features of lactational differentiation by the production of β-casein protein in virgin transgenic mammary glands. This implies that β-casein expression can be dissociated from the morphological changes, and may be directly related to alterations in the integrity of the basement membrane of mammary epithelial cells.

The experiments presented in this fellowship were designed to determine if *MAT* expression can contribute to mammary tumor invasion and metastasis. MMTV-*neu* transgenic mice provide a reasonable model of human breast cancer, spontaneously developing adenocarcinomas with metastatic potential. The introduction of MMTV-driven *MAT* into these tumors in bigenic mice resulted in a small but not statistically significant increase in the percentage of mice with lung metastases. The most striking effect of *MAT* in these mice was the surprising and dramatic acceleration in the onset of tumors, accompanied by an increase in the percent of animals with tumors at a defined endpoint. Since metastatic lesions arise from the primary tumor, the observed increase in metastatic lesions may be an indirect result of the increase in the number of animals with primary tumors, or the duration of time these animals contained tumors. We also noted that the *MAT* transgene is expressed only sporadically in the periphery of advanced lesions, perhaps due to the loss of differentiation characteristics in these adenocarcinomas and reduction in expression of the MMTV promoter. Dispite this caveat, we conclude that, in our model system, there is no direct evidence for a role for *MAT* in the metastatic spread of mammary adenocarcinomas.

Our observation that overexpressing *MAT* in the MMTV-*neu* transgenic model system accelerates the onset of mammary tumor formation by 13 weeks, which represents approximately 1/3 the lifespan of these animals, is very striking. These data, the presence of *MAT* in a high percentage of preneoplastic human breast lesions (Heppner et al, 1996), and the appearance of HANs in *MAT*-expressing multiparous animals, leads us to speculate that *MAT* plays an important role in the early induction of mammary tumors.

The mechanism underlying the tumor-enhancing property of *MAT* expression in MMTV-*neu* mice was addressed in this study. We hypothesized that MAT activity could result in an increase in the availability of soluble ErbB receptor ligands, either through cleavage of membrane precursors or release of ligand from matrix components. We tested the role of Neu-related signal transduction in *MAT*-accelerated tumorigenesis by comparing the levels, the ability to heterodimerize, and the activation of these receptors as determined by phosphorylation state of ErbB receptors. We observed no obvious differences in these parameters in MMTV-*neu* versus MMTV-*MAT/neu* tumors or in mammary glands that were prone to develop tumors. Rather, both groups of mammary tumors contain deletions within the *neu* transgene at approximately the same frequency. These data imply that, although the presence of MAT does not alter signalling through the ErbB family of receptors, the addition of *MAT* accelerates the deletions that occur within the *neu* transgene to induce the formation of mammary tumors.

The appearance of HANs in the MMTV-*MAT* animals and the acceleration of MMTV-*neu*-induced tumors in MMTV-*MAT/neu* transgenic mice suggests that the expression of *MAT* in the mammary epithelium contributes to early-stage mammary tumorigenesis. Since *MAT* has been observed in benign and preneoplastic breast lesions, as well as in apparently normal mammary epithelium, these results suggest that inhibition of MAT activity in individuals with an elevated risk for mammary carcinoma may provide a protective advantage. Additional studies with synthetic MMP inhibitors and genetically-manipulated mice offer the opportunity to test the potential of this strategy in the prevention of malignant breast disease.

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FIGURE LEGENDS

Figure 1. Expression of the human MAT transgene in developing mammary tissue. Northern analysis of poly A+ selected RNA (4μg) from nontransgenic and transgenic female mammary tissue at various weeks during mammary development. Blots were probed with a 1.1kb ^{32}P-labelled human MAT cDNA probe (HMAT) and a cyclophilin (1B15) cDNA probe was used to control for RNA loading. A) Expression of MAT transgene is present at relatively high levels in selected samples from line #3 and #42 and absent in the nontransgenic littermates. B) Line #1 and line #22 express the MMTV-ActMAT transgene, while nontransgenic littermates do not express the transgene. C) Isolation of intact human MAT mRNA from both MMTV-InMAT transgenic lines #2 and #4 was not possible after several attempts. Degraded human MAT mRNA as shown was consistently isolated in both lines of transgenic mammary glands.

Figure 2. Localization of human MAT protein by immunohistochemistry. Representative sections from developing transgenic (A, B, C) and nontransgenic (D) mammary glands were probed with an affinity-purified polyclonal antibody against a human MAT peptide. Staining of human MAT is in the cytoplasm of the mammary ducts from MMTV- *MAT* wildtype line #3 (A) and to a lesser extent in MMTV-Act*MAT* line #22 (B) developing glands (arrows). No specific staining was detected in MMTV-In*MAT* line #2 transgenic (C) or nontransgenic (D) mammary glands. Magnification = 50X.

Figure 3. Morphological appearance of developing mammary glands. Iron hematoxylin stained whole mounts of inguinal mammary glands from MMTV-*MAT* line #3 transgenic (A and B) and nontransgenic (C and D) female virgin animals. Glands were removed at 14 weeks (A and C) and 16 weeks (B and D) of mammary gland development. Magnification = 2.5X (A and C) and 6.4X (B and D).

Figure 4. Casein expression in virgin MMTV-*MAT* mammary glands. Wildtype MMTV-*MAT* line #3 transgenic mammary glands (A and B) at 6 weeks of age were positive for β-casein expression using immunohistochemistry, while nontransgenic control mammary glands (C and D) were negative. Panels A and C were taken using 16X magnification and panels B and D were taken using 32X magnification.

Figure 5. MMTV-*MAT* expression of MMTV-*MAT/neu* mammary tumors. Localization of MAT in the MMTV-*MAT/neu* mammary tumors to the periphery and border of the tumor (arrows). T indicates the primary mammary tumor (50X magnification).

Figure 6. ErbB receptor expresion in the MMTV-*MAT/neu* and MMTV-*neu* mammary tumors. A) Co-immunoprecipitation of the ErbB family of receptors in MMTV-*MAT/neu* and MMTV-*neu* mammary tumor extracts. Immunoprecipitation of the EGFR, followed by western blot for the Neu protein. B and C) Immunoprecipitation and western blot analysis of erbB receptors in mammary tumor extracts from MMTV-*MAT/neu* and MMTV-*neu* animals. Each ErbB receptor [A) EGFR and B) Neu] was specifically immunoprecipitated from 300μg of mammary tumor extracts then western blotted and analyzed for the levels of phosphorylation (p-Tyr), or for the presence of the immunoprecipitated receptor to control for the loading of protein. Several mammary tumor samples were assayed for both transgenic groups with identical results observed. Two representative samples are shown for each group.

Figure 7. Multiparous mammary gland phenotype in the MMTV-*MAT* transgenic animals. Whole mount staining of inguinal mammary glands taken from multiparous transgenic (A) and nontransgenic (B) animals. Whole mounts shown are representative of several multiparous females animals analyzed. Note the appearance of HANs (H).

PUBLICATIONS (Resulting from this work)

Hulboy, D.J., Rudolph, L.A., and Matrisian, L.M. Matrix metalloproteinases as mediators of reproductive functions. *Molecular Human Reproduction*, 3:27-45, 1997. (Review)

Rudolph-Owen, L.A., Cannon, P, and Matrisian, L.M. Overexpression of the metalloproteinase matrilysin results in premature mammary gland differentiation and male infertility. Submitted.

Rudolph-Owen, L.A., Muller, W.J., and Matrisian, L.M. The matrix metalloproteinase matrilysin influences early-stage mammary tumorigenesis. In preparation.

Rudolph-Owen, L.A. and Matrisian, L.M. Tissue remodeling in normal and neoplastic mammary gland through matrix metalloproteinases. *Journal of Mammary Gland Biology and Neoplasia*. In preparation. (Review)

MacDougall, J.R., Rudolph-Owen, L.A., Arteaga, C.L., and Matrisian, L.M. The matrix metalloproteinase matrilysin is regulated by both positive and negative factors in breast cancer progression. In preparation.

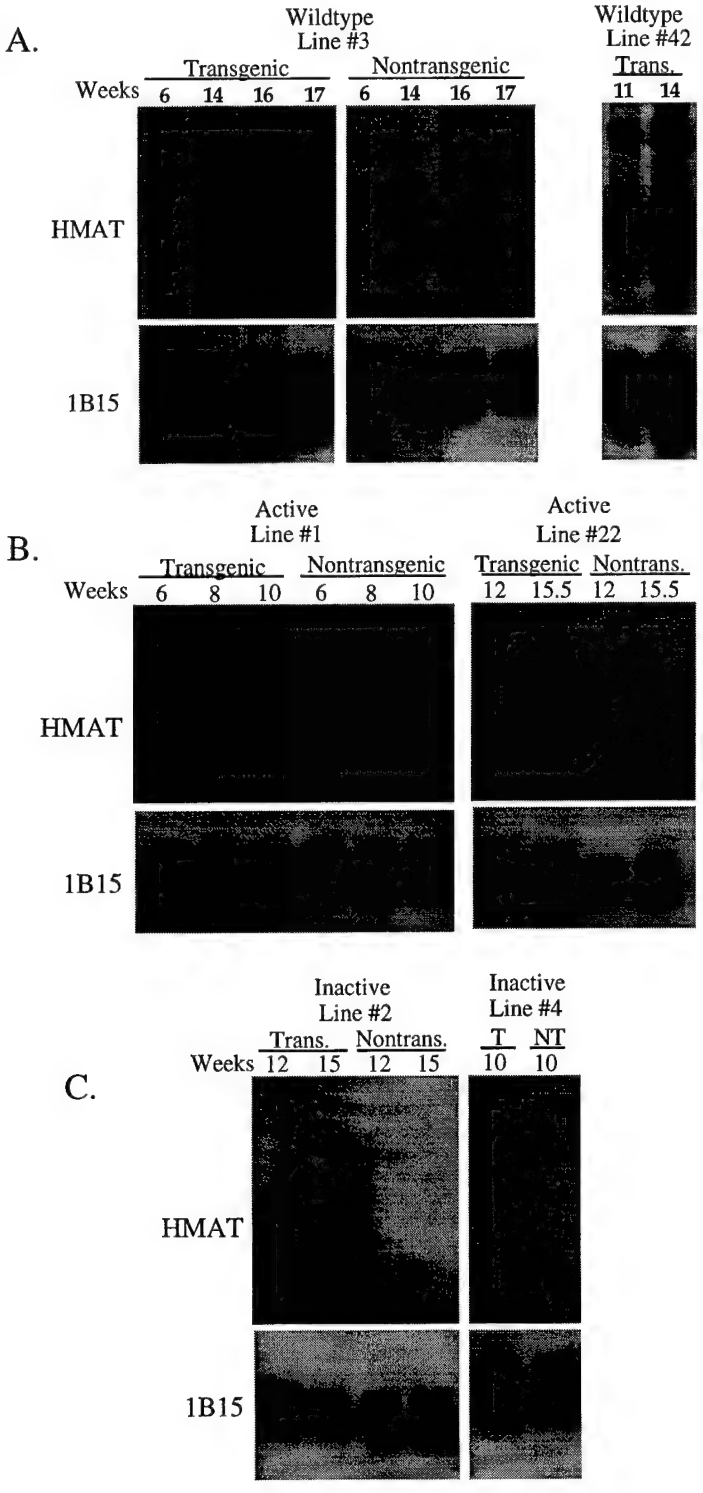
ABSTRACTS

Rudolph, L.A. and Matrisian, L.M. Alterations resulting from the overexpression of the matrix metalloproteinase matrilysin in the murine mammary gland. Presented at the Mammary Gland Biology Gordon Conference, New London, NH, June 1995.

Rudolph, L.A. and Matrisian, L.M. Overexpression of human matrilysin results in the aberrant development of the testis and mammary glands and accelerates mammary tumor formation. Presented at Protease and Protease Inhibitors Meeting, Panama City Beach, FL, March 1996.

Rudolph, L.A. and Matrisian, L.M. Human matrilysin overexpression results in higher frequency and accelerated growth rates of mammary tumors in transgenic animals. *Clinical and Experimental Metastasis*, 14 (Supp. 1):64, 1996. Presented at the Sixth International Congress of the Metastasis Research Society, Aula of the University of Gent, Gent, Belgium. Sept., 1996.

MacDougall, J.R., Rudolph, L.A., Arteaga, C., and Matrisian, L.M. Expression of the matrix metalloprotienase matrilysin is regulated by both positive and negative factors in breast cancer cells. (Vol 38; Abstract #2717) Presented at the American Association for Cancer Research, 88th Annual Meeting, San Diego, CA, April 1997.


A.
Wildtype
Line #3
Transgenic
Nontransgenic
Weeks
6 14 16 17
6 14 16 17
Wildtype
Line #42
Trans.
11 14
HMAT
1B15
B.
Active
Line #1
Transgenic
Nontransgenic
Weeks
6 8 10 6 8 10
Active
Line #22
Transgenic
Nontrans.
12 15.5 12 15.5
HMAT
1B15
C.
Inactive
Line #2
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Nontrans.
Weeks
12 15 12 15
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Line #4
T
NT
10 10
HMAT
1B15

Figure 1

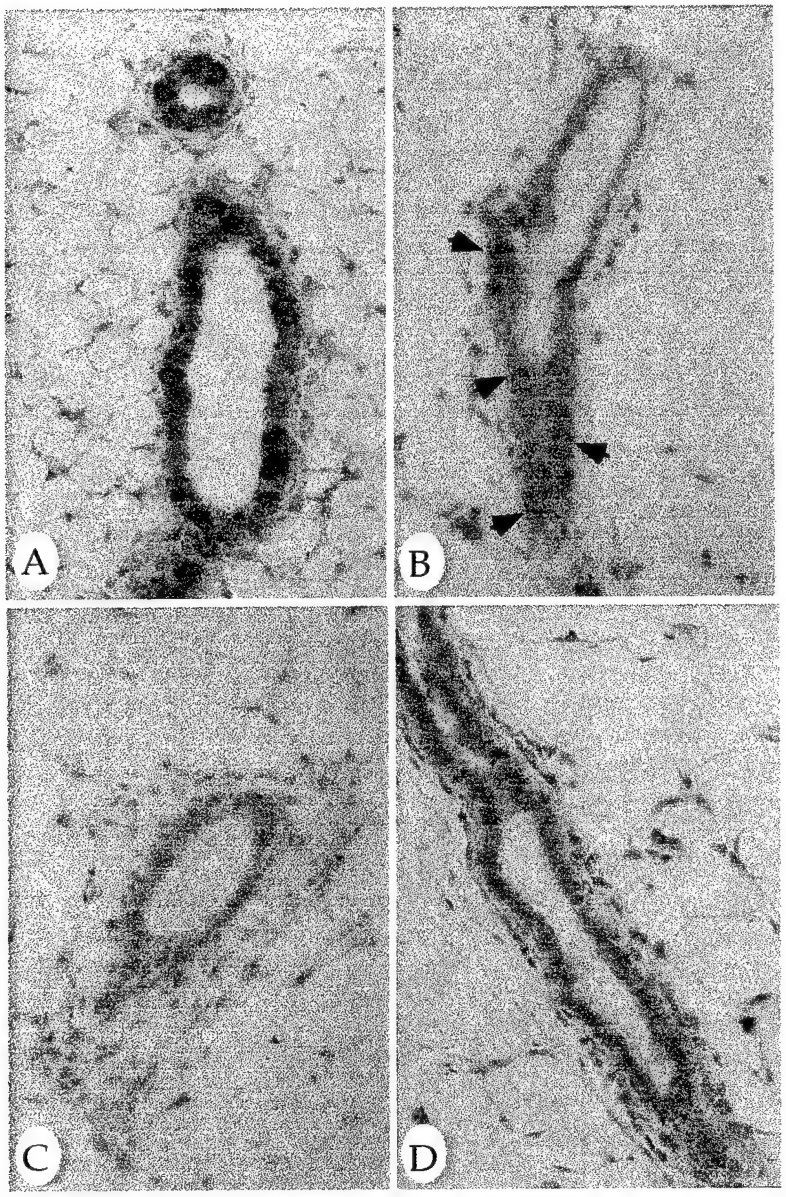


Figure 2

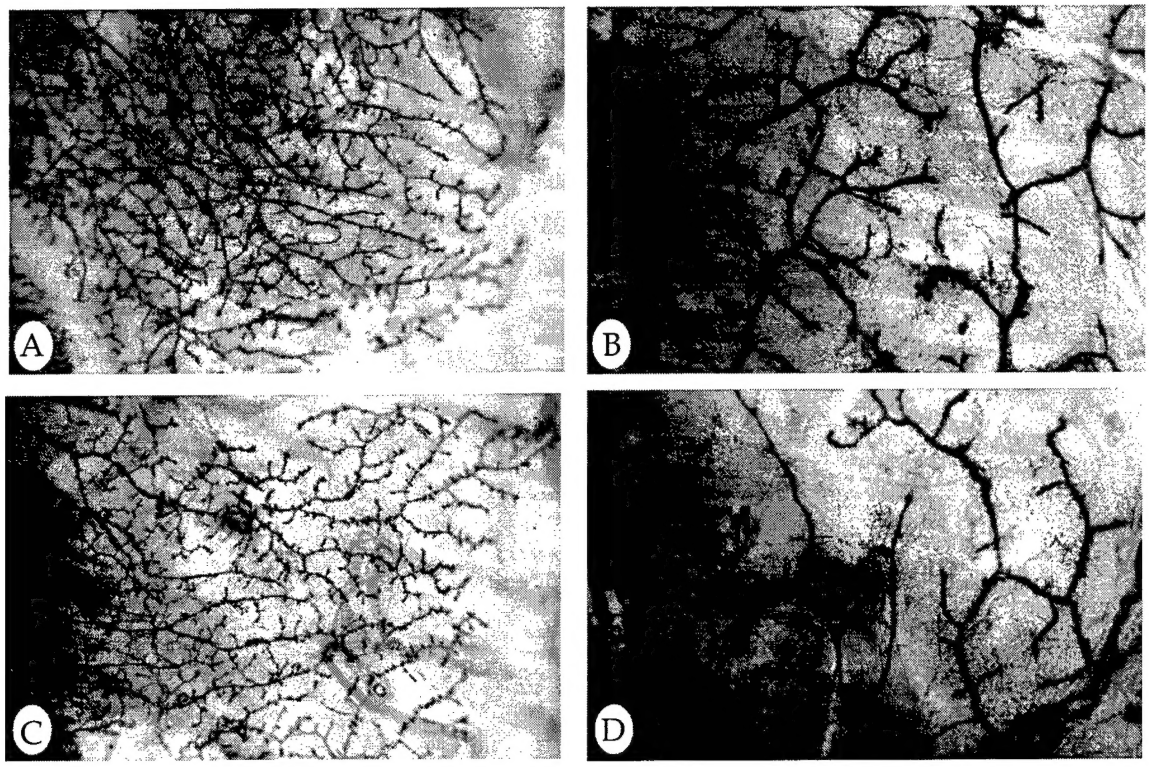


Figure 3

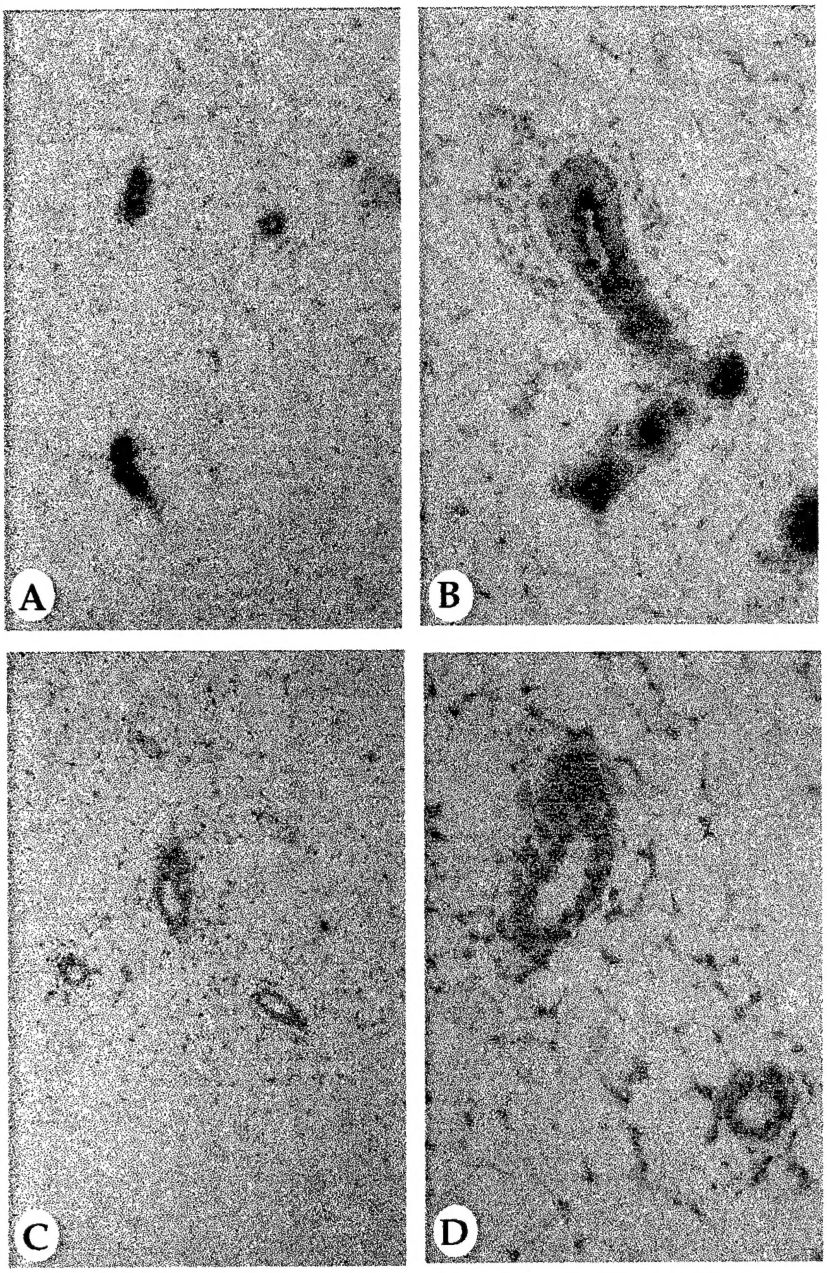


Figure 4

Figure 5

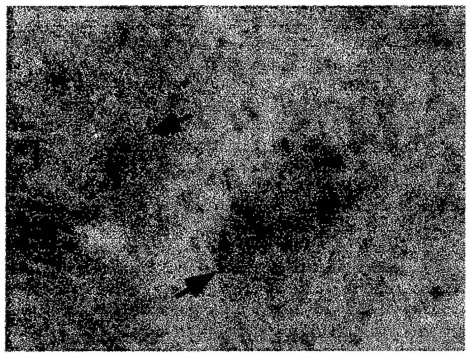
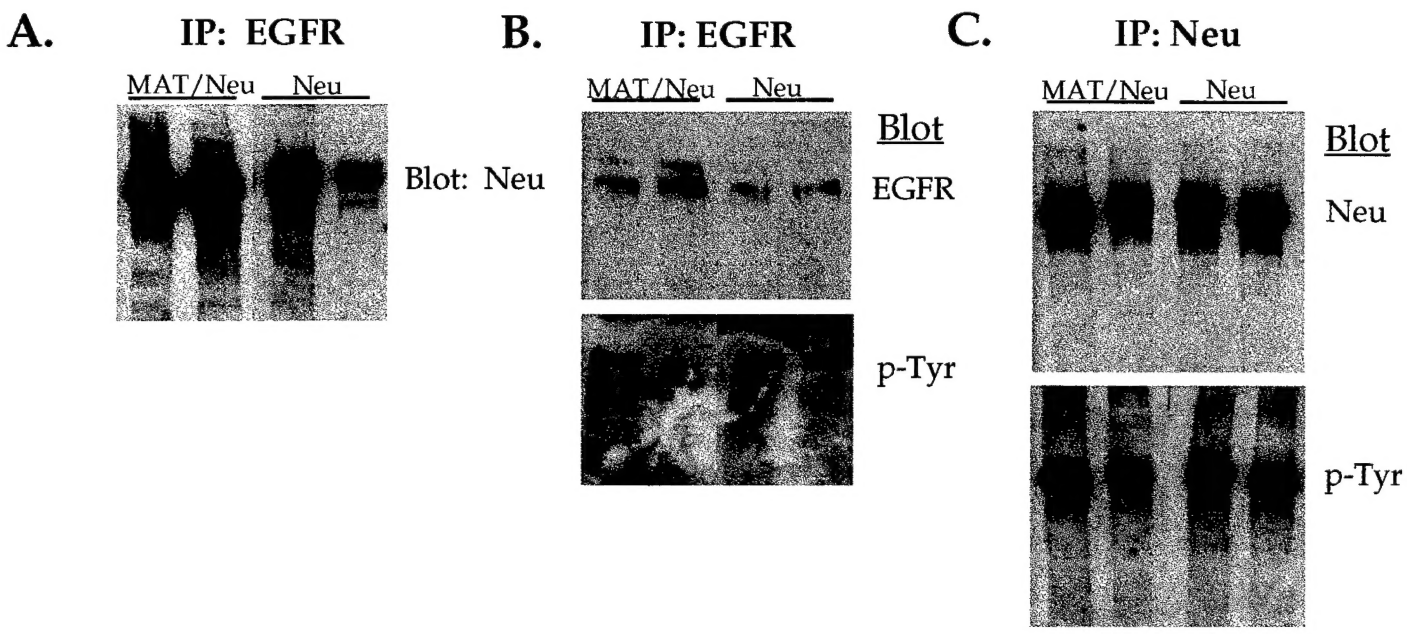


Figure 6



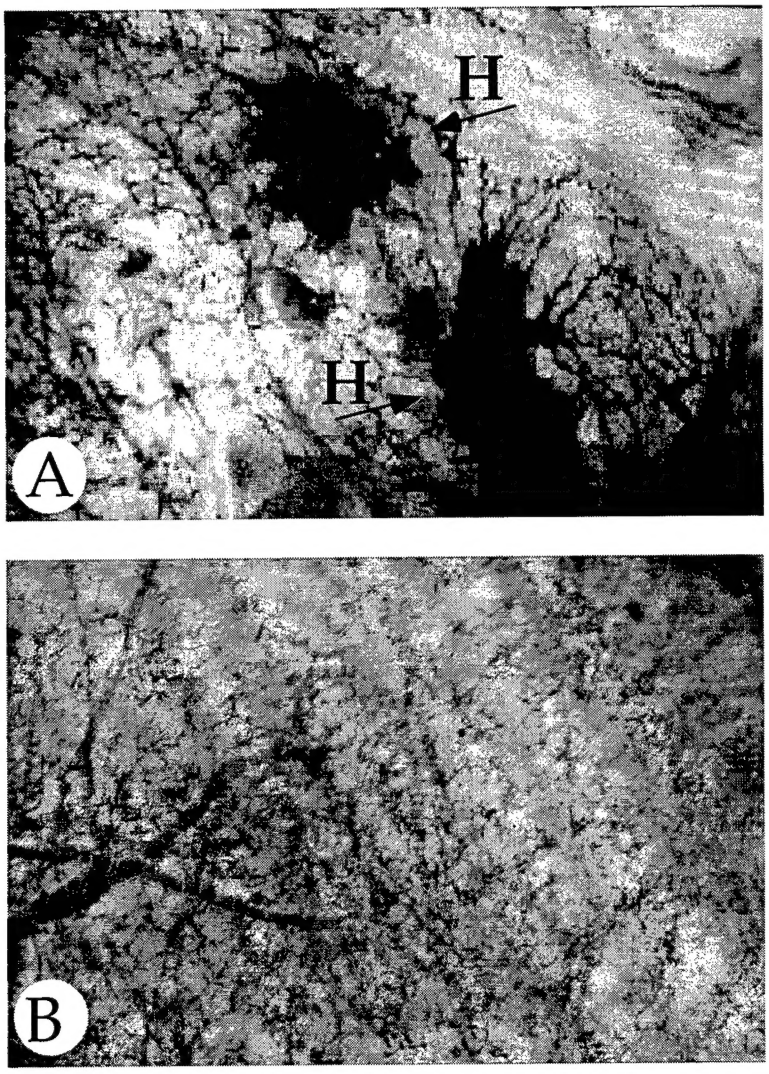


Figure 7